Effect of Baicalin on inflammatory mediator levels and microcirculation disturbance in rats with severe acute pancreatitis

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Abstracts

Objective: To investigate the effect of Bacailin on inflammatory mediator levels and microcirculation disturbance in severe acute pancreatitis (SAP) rats and explore its therapeutic mechanism on this disease.

Methods: SAP model rats were randomly divided into model control group and Baicalin treated group, 45 rats in each group. The same number of normal rats were included in sham-operated group. These groups were further subdivided into 3 h, 6 h and 12 h subgroups, respectively (15 rats in each subgroup). At 3, 6 and 12 hours after operation, rats were killed to conduct the following experiments: (1) to examine the mortality rates of rats, the ascites volume and pancreatic pathological changes in each group; (2) to determine the contents of amylase, PLA_2, TXB_2, PGE_2, PAF and IL-1β in blood as well as the changes in blood viscosity.

Results: (1) Compared to model control group, treatment with Baicalin is able to improve the pathological damage of the pancreas, lower the contents of amylase and multiple inflammatory mediators in blood, decrease the amount of ascitic fluid and reduce the mortality rates of SAP rats ($P<0.01$, $P<0.01$ or $P<0.001$); (2) at 3 hours after operation, the low-shear whole blood viscosity (1/s)(mPa.s) in Baicalin treated group was significantly lower than that in model control group ($P<0.01$); at 12 hours after operation, both the high-shear (200/s) (mPa.s) and low-shear whole blood viscosity (1/s) (mPa.s) in Baicalin treated group were also significantly lower than those in model control group ($P<0.01$ for all).

Conclusion: Baicalin, as a new drug, has good prospects in the treatment of SAP since it can exert therapeutic effects on this disease through inhibiting the production of inflammatory mediators, lowering blood viscosity, improving microcirculation and mitigating the pathological damage of the pancreas.

Keywords: severe acute pancreatitis (SAP); rats; Baicalin; inflammatory mediators; microcirculation

1. Introduction

In recent years, the roles of microcirculation disturbance in the pathogenesis of severe acute pancreatitis (SAP) have attracted extensive attention [1,2]. Microcirculation disturbance refers to the morphological alterations and functional disturbance of blood vessels or blood flow at microcirculatory level. Hemorheological changes are one of the most important factors that can induce microcirculation disturbance. Even in the early stage of SAP, an obvious disturbance of pancreatic microcirculation is present. Therefore, it has important significance to improve pancreatic microcirculation in the treatment of SAP [3]. In the present study, we investigate the effect of Baicalin on inflammatory mediator levels and

microcirculation disturbance in SAP rats and explore its therapeutic mechanism, thereby providing a theoretical basis for treating this disease with Baicalin.

2 Materials and methods

2.1 Main materials: Clean grade healthy male Sprague-Dawley (SD) rat in 250 - 300 g of body weight purchased from the Experimental Animal Center of Medical School, Zhejiang University,China. Sodium taurocholate and sodium pentobarbital purchased from USA Sigma Company; Octreotide purchased from Swiss pharmaceutical company Novartis; 1% Baicalin injection (China national invention patent number ZL200310122673.6) prepared by the first author with 310 mmol/L osmotic pressure; The full automatic biochemical analyzer was used to determine the plasma amylase level (unit of measure: U/L); Thromoboxane B_2 immunoassay kit (catalog No.DE0700; unit of measure:pg/mL) and PGE_2 immunoassay kit (catalog No.DE0100; unit of measure:pg/mL) were purchased from the RδD system Ins (USA). PAF immunoassay kit (unit of measure:ng/L) and IL-1β immunoassay kit (unit of measure:ng/L) were purchased from the Hangzhou Haotian Biotechnology Co. LTD (China). These assays were conducted according to the instructions provided in the kits. Blood viscosity was determined using SA-6000 automatic hemorheological testing instrument, which was provided by the Beijing Success Technology Development Co., LTD (China).

2.2Methods

2.2.1. Animal grouping: Ninety SAP model rats were prepared with 3.5% sodium taurocholate and randomly divided into model control group and Baicalin treated group, 45 rats in each group. The same number of normal rats were included in sham-operated group, which only received laparotomy. These groups were further subdivided into 3 h, 6 h and 12 h subgroups, respectively (15 rats in each subgroup).

2.2.2 Preparation of SAP model and procedure of treatment: Fast but water restraint was imposed on all rat groups 12h prior to the operation. The rats were anesthetized by intraperitoneal injection of 2% sodium pentobarbital (0.25ml/100g) after which lay and fixed the rats, and performed the routine shaving, disinfection and draping. First established the right external jugular vein transfusion passage used the microinfusion pump for continuous transfusion (1ml/h/100g) and then used 3.5% sodium taurocholate to prepare SAP model. In model control group: After entering abdomen via median epigastrium incision, confirmed the bile-pancreatic duct and hepatic hilus common hepatic duct, disclosed the pancreas, identified the duodenal papilla inside the duodenum duct wall, and then used a No.5 needle to drill a hole in the mesenterium avascular area. After inserting a segmental eqidural catheter into the duodenum cavity via the hole, inserted the bile-pancreatic duct toward the direction of papilla in a retrograde way, used the microvascular clamp to nip the duct head temporarily and meanwhile used another microvascular clamp to temporarily occlude the common hepatic duct at the

confluence of hepatic duct. After connecting the anaesthetic tube end with the transfusion converter, transfused 3.5% sodium taurocholate (0.1ml/100g) by retrograde transfusion via the microinjection pump (made by Zhejiang University) at the speed of 0.2ml/minute. Stayed for 4 to 5 minutes after injection and removed the microvascular clamp and epidural catheter. After checking for bile leakage, sutured the hole in the duodenum lateral wall. Used the disinfected cotton ball to absorb up the anaesthetic in the abdominal cavity and close the abdomen. Sham-operated group after receiving abdomen opening was only performed pancreas and duodenum turning over and finally abdomen closing. In Baicalin treated group: The animal experiments of 1% Baicalin injection have been completed including the acute toxicity test and SAP rat treatment by small, middle and large dose. The large dose can achieve the best therapeutic effect (dose is 10mg/h/100g) and the dosage referred to the result of the previous preliminary experiment. 10 minutes after successful modeling, Baicalin treatment group was first injected 5% Baicalin injection 10mg/100g via external jugular vein passage followed by continuous intravenous administration (10mg/h/100g) by microinfusion pump; The dosage of Baicalin has been proved as effective dosage in the previous preliminary experiment. Both of sham-operated group and model control group were injected saline of equivalent volume at the corresponding time points after operation.

2.2.3. Parameters determined: at 3, 6 and 12 hours after operation, the mortality rates of rats in each group were observed. These rats were then killed and subjected to examination of the ascites volume and the pathological changes of the pancreas

as well as the score of pathological changes under light microscopy [4]. The blood was then collected to determine the contents of amylase, TXB_2, PGE_2, PAF and IL-1β in blood. At 3 and 12 hours after operation, blood viscosity, including high-shear whole blood viscosity (200/s) (mPa.s), whole blood viscosity (30/s) (mPa.s) and low-shear whole blood viscosity (1 or 5/s) (mPa.s), were measured.

2.2.4. Statistical analysis: all data were analyzed using the SPSS13.0 software. Because the comparisons of the pathological scores of the pancreas, the contents of PAF, amylase, TXB_2, PGE_2, PAF and IL-1β in blood as well as the ascites/body weight ratio belong to non-parameter test, the Kruskal-Wallis test was used for multiple comparisons among the three groups. The Mann-Whitney U test was used for pair-wise comparisons. The correlations among each parameter were analyzed using the Spearman correlation test. Since all four blood viscosity parameters meet the normal distribution and should be subjected to parameter test, the ANOVA test was used for multiple comparisons of these parameters among the three groups. The Bonferroni test was used for pair-wise comparisons. The correlations among each parameter were analyzed using the Spearman correlation test. Statistical significance was concluded if the *P* value was less than or equal to 0.05.

3 Results

3.1. Comparison of mortality rate: in sham-operated group, no rats died at 3, 6 and 12 hours after operation. In model control group, one and four rats died at 6 and 12 hours after operation, respectively. In Baicalin treated group, one rat died at 12 hours after operation. At 12 hours after operation, the mortality rate of rats in sham-operated group and treated group were significantly lower than that in model control group ($P = 0.05$).

3.2. Comparison of pathological scores of the pancreas, ascites volume and contents of inflammatory mediators in blood: the results were shown in Tables 1 and 2.

3.2.1. At three hours after operation: the pathological scores of the pancreas, the contents of PAF, amylase, TXB_2, PGE_2 and IL-1β in blood as well as the ascites/body weight ratio in sham-operated group were significantly lower than those in model control group and Baicalin treated group ($P<0.001$ for all). The contents of plasma amylase and the ascites/body weight ratio in treated group were significantly lower than those in model control group ($P<0.005$). Besides, the content of serum IL-1β in the former group was also significantly lower than that in the latter group ($P<0.01$).

3.2.2. At six hours after operation: the pathological scores of the pancreas, the contents of PLA_2, PAF, amylase, TXB_2 and PGE_2 in blood as well as the ascites/body weight ratio in sham-operated group were significantly lower than those in model control group ($P<0.001$ for all). The pathological scores of the pancreas, the contents of PLA_2, PAF, amylase, TXB_2, PGE_2 and IL-1β in blood as well as the ascites/body weight ratio in sham-operated group were also significantly lower than those

in Baicalin treated group ($P<0.001$ for all). The content of TXB_2 in blood in treated group was significantly lower than that in model control group ($P<0.05$). Besides, the ascites/body weight ratio and the content of TXB_2 in blood in the former group were also significantly lower than those in model control group ($P<0.001$).

3.2.3. At twelve hours after operation: the pathological scores of the pancreas, the contents of PLA_2, PAF, amylase, TXB_2, PGE_2 and IL-1β in blood as well as the ascites/body weight ratio in sham-operated group were significantly lower than those in model control group ($P<0.001$ for all). The pathological scores of the pancreas, the contents of PLA_2, PAF, amylase, TXB_2, and PGE_2 in blood as well as the ascites/body weight ratio in sham-operated group were also significantly lower than those in Baicalin treated group ($P<0.001$ for all). The pathological scores of the pancreas, the contents of amylase and PLA_2 as well as the ascites/body weight ratio in treated group were significantly lower than those in model control group ($P<0.01$, $P<0.01$, $P<0.001$ and $P<0.05$, respectively).

3.3. Correlation analysis results (Spearman correlation):

3.3.1. Model control group: at 12 hours after operation, a negative correlation between the contents of amylase and PGE_2 in blood was noted ($R=-0.678$, $P<0.05$).

3.3.2. Sham-operated group: at 12 hours after operation, a positive correlation between the content of amylase in blood and the ascites/body weight ratio was noted ($R=0.533$, $P<0.05$).

3.3.3. Baicalin treated group: at 6 hours after operation, a positive correlation between the pathological scores of the pancreas and the content of amylase in blood was noted ($R=0.579$, $P<0.05$); at 12 hours after operation, a positive correlation between the content of PAF in blood and the ascites/body weight ratio was observed ($R=0.618$, $P<0.05$).

3.4. Comparison of blood viscosity parameters: the results were shown in Table 3.

3.4.1. At three hours after operation: the low-shear whole blood viscosity in model control group was significantly higher than that in sham-operated group ($P<0.01$); the whole blood viscosity and low-shear whole blood viscosity (5 or 1/s) (mPa.s) in model control group were significantly higher than those in sham-operated group ($P<0.001$). The whole blood viscosity (30/s) (mPa.s) and low-shear whole blood viscosity (1/s) (mPa.s) in treated group were significantly higher than those in sham-operated group ($P<0.05$) while the low-shear whole blood viscosity (1/s) (mPa.s) in treated group was significantly lower than that in model control group ($P<0.01$).

3.4.2. At twelve hours after operation: the high-shear whole blood viscosity (200/s) (mPa.s), whole blood viscosity (30/s) (mPa.s) and low-shear whole blood viscosity (1/s) (mPa.s) in model control group were significantly higher than those in sham-operated group ($P<0.001$). The high-shear whole blood viscosity (200/s) (mPa.s) and low-shearwhole blood viscosity (5/s) (mPa.s) in treated group were significantly higher than those in sham-operated group ($P<0.01$ and $P<0.05$, respectively). The high-shear whole blood viscosity (200/s) (mPa.s) and low-shear whole blood viscosity (1/s) (mPa.s) in

treated group were significantly lower than those in model control group ($P<0.01$).

3.5. Gross pathological changes and pathological changes under light microscopy of the pancreas

3.5.1. Sham-operated group

3.5.1.1. Gross pathological changes: except that there was a small amount of light yellow ascites present in the abdominal cavity, no other obvious pathological changes were observed. The pancreas showed intact structure and light yellow color. At all time points after operation, no obvious abnormality was seen in the pancreas as well as peripancreatic fat and omenta.

3.5.1.2. Pathological changes under light microscopy: the pancreas was normal in most rats and showed intact structure. Mild interstitial edema was seen in few cases. Inflammatory cell infiltration was occasionally viewed.

3.5.2. Model control group

3.5.2.1. Gross pathological changes: five minutes after operation, the pancreas showed extensive subcapsular hemorrhage and necrosis, particularly prominent in the pancreatic tail. At three hours after operation, pancreatic congestion and edema, some of which showed jelly-like hemorrhage or necrosis, were obviously seen; a small amount of light red ascites was present. At 6 and 12 hours after operation, the pancreas showed extensive congestion and necrosis as well as obvious edema; a moderate number of saponified spots were visible in peripancreatic epiploon and peritoneum; a large amount of

light red or red bloody ascites was present in the abdominal cavity.

3.5.2.2. Pathological changes under light microscopy: interstitial edema, few focal or lytic necrotic spots and inflammatory cell infiltration were present in the pancreatic head; the pancreatic tail showed more obvious interstitial edema, larger focal or lytic necrotic spots as well as more severe inflammatory cell infiltration; the extent and scope of the necrosis in the pancreatic tail were more significant than those in the pancreatic head; the pathological changes were aggravated with the increase of postoperative duration.

3.5.3. Baicalin treated group:

3.5.3.1. Gross pathological changes: at 3 hours after operation, the pancreas showed congestion and necrosis; the hemorrhage was milder than that in model control group. At 6 and 12 hours after operation, the scope of hemorrhage in the pancreas was relatively restricted; the extent of pancreatic edema and necrosis was milder than that in model control group; the majority of ascites became slightly bloody; the amount of ascites was obviously lower than that in model control group; the number and density of saponified spots also decreased.

3.5.3.2. Pathological changes under light microscopy: patchy necrosis still could be seen in the pancreas but was distributed in a smaller area; the cellular structure in the pancreas was clearer than that in model control group; the extent of interstitial red blood cell exudation, edema and inflammatory cell infiltration was mitigated.

4. Discussion

SAP is an acute abdomen characterized by dangerous clinical manifestations and high mortality rate [5-7]. In recent years, some studies have found that, during the development and progression of SAP, the premature activation of pancreatic enzymes in acinar cells and excessive inflammatory reaction can induce the production of a large number of cytokines and vasoactive substances that are able to, directly or indirectly, give rise to the decrease in local blood flow, blood flow velocity, leukocyte adhesion and capillary density as well as the increase in capillary permeability, thereby inducing microcirculation disturbance [8, 9]. In turn, microcirculation disturbance is one of the important factors that can induce the production of inflammatory mediators. Thus, both microcirculation disturbance and inflammatory mediators serve as causes and effects working to form the viscous cycle [10]. For these reasons, it is of important significance to improve microcirculation disturbance and reduce inflammatory mediator-induced damage in the therapy of SAP.

Baicalin, the main effective ingredient of scutellaria baicalensis georgi, can be administered via veins and is very cheap. It has antibacterial and anti-inflammatory effects, and is able to inhibit platelet aggregation, scavenge oxygen free radicals, suppress thrombin-induced transformation of fibrinogen into fibrin, decrease the production of endotoxin and prevent endotoxemia-induced DIC. Moreover, baicalein, the metabolite of Baicalin, shows strong effect in suppressing the

activities of pancreatic enzymes [11]. These pharmacological effects, similar to those of octreotide (a somatostatin analogue), can block the development of SAP in many aspects. Since the pharmacological effects of Baicalin are even more diverse, it is theoretically feasible to treat SAP with Baicalin.

When SAP develops, PAF can not only induce a series of inflammatory reactions through acting upon leukocytes but also cause pancreatic microcirculation disturbance through increasing vascular permeability [12, 13]. In this study, we found that, at all time points after operation in model control group and at 6 and 12 hours after operation in Baicalin treated group, the contents of PAF were significantly higher than those in sham-operated group ($P<0.001$ for all), indicating that the development of SAP can enhance the release of PAF and thereby induce microcirculation disturbance. We also found that, at 6 and 12 hours after operation in Baicalin treated group, the contents of PAF were significantly lower than those in model control group, suggesting that Baicalin is able to lower the levels of PAF and thereby improve microcirculation disturbance. However, no statistically significant difference was observed because sample size was too small, suggesting that Baicalin has a weak effect in lowering the levels of PAF.

The leukocyte-endothelial cell interactions and microcirculation alterations are two crucial factors that are responsible for the development of SAP [14], during which phospholipase A_2 (PLA_2), whose levels can be used as a marker for evaluation of the severity of SAP, plays an important role. When SAP develops, the level of PLA_2 rises abnormally, thus

accelerating the synthesis of arachidonic acid and inducing the production of large amounts of thromboxane A_2 (TXA_2) and prostaglandin E_2 (PGE_2). TXA_2 can potently contract blood vessels, promote platelet aggregation, recruit leukocytes and increase microvascular permeability [15]. Because TXA_2 is degraded rapidly, TXB_2 as a stable metabolite of TXA_2 is usually detected to reflect the levels of TXA_2. PGE_2, secreted by endothelial cells, is able to expand capillary, increase blood supply to tissues, protect cells and thereby antagonize the effect of TXA_2. In this study, correlation test showed that there was a negative correlation between the contents of amylase and PGE_2 ($R=-0.678$, $P<0.05$). The imbalance in the ratio between the levels of TXB_2 and PGE_2 has more severe effects than the alteration of only one factor. In SAP, the dysfunction of the arachidonic acid metabolic network can upregulate the levels of TXB_2. As a consequence, the imbalance in the ratio between the levels of TXB_2 and PGE_2 can promote platelet aggregation and thrombosis [16, 17], and thereby induce microcirculation disturbance as well as pancreatic and extrapancreatic ischemia and necrosis. Most researchers believe that the increase in the levels of blood TXB_2 or the ratio between the levels of TXB_2 and PGE_2 may promote the progression of SAP. In this study, we found that the levels of TXB_2 and PGE_2 in model control group were higher than those in sham-operated group. Moreover, the elevating amplitude of TXB_2 level was much greater than that of PGE_2 level, thereby causing the imbalance in the ratio between them and aggravating organ injury. Considering that Baicalin could decrease the ratio between the levels of TXB_2 and PGE_2, it could therefore improve pancreatic injury.

IL-1β is able to activate neutrophils and induce microcirculatory thrombosis and tissue injury. Some experiments have proved that the contents of IL-1β are positively correlated with the mortality rates of rats [18]. Paszkowski et al. [19,20] have found that IL-1β mRNA is expressed in normal lung tissues. In SAP, the expression of IL-1β mRNA is significantly enhanced, and the expression levels of IL-1β mRNA are parallel to the severity of SAP. In the present study, our results showed that, at all time points after operation, the contents of IL-1β in model control group were significantly higher than those in sham-operated group ($P<0.001$), and the levels of IL-1β are positively correlated with the severity of the pathological damage of the pancreas. After treatment with Baicalin, the contents of serum IL-1β in treated group were significantly lower than those in model control group ($P<0.01$ or $P<0.001$) at 3 and 6 hours after operation. Moreover, the pathological damage of the pancreas was mitigated. These results indicate that Baicalin is able to lower the contents of IL-1β in SAP rats and have therapeutic effect on SAP.

Numerous studies have shown that the levels of the above-mentioned inflammatory mediators are upregulated in most cases of SAP and can be used as important parameters for evaluation of the severity and prognosis of SAP [21-26], which is consistent with the results obtained in this study. The levels of these inflammatory mediators and pancreatic pathological scores in Baicalin treated group were obviously lower than those in model control group, suggesting that Baicalin is able to lower the levels of inflammatory mediators and mitigate the pathological damage of the pancreas.

Blood viscosity test is one of the most direct and convenient ways to study microcirculation. In the present study, we found that the high-shear whole blood viscosity, whole blood viscosity and low-shear (1 or 5/s) whole blood viscosity (mPa.s) in model control group were significantly higher than those in sham-operated group ($P<0.01$ or $P<0.001$), indicating that SAP model rats induced with sodium taurocholate showed obvious hemorheological abnormalities, which were mainly manifested as an obvious increase in erythrocyte aggregation index, viscous component, elastic component and elastic modulus of the peripheral blood at low shear rates, strong RBC aggregation and a decrease in the deformability of cells. These changes were gradually aggravated with the progression of the disease [27]. We also found that the pancreas of rats in model control group showed hemorrhage and necrosis, and a moderate number of saponified spots were visible in peripancreatic epiploon and peritoneum. After treatment with Baicalin, blood viscosity was obviously improved, the scope of pancreatic hemorrhage and necrosis was relatively limited, and the number of saponified spots decreased. These results suggest that Baicalin could mitigate the pathological injury present in SAP through improving the hemorheology of rats. When SAP develops, microcirculation disturbance can induce the increase of pancreatic capillary permeability, thereby causing hemorheological changes such as hemoconcentration, the increase in hematocrit and blood viscosity, etc. [28]. In this study, at all time points after operation, blood viscosity parameters determined in model control group were obviously greater than those in sham-operated group ($P<0.01$ or $P<0.001$), indicating that hemorheological abnormality and

microcirculation disturbance are present in SAP. These abnormal alterations can induce the damage of vascular endothelial cells, increase the resistance of blood flow, promote the stagnation of blood flow and thrombosis, and result in hypoxia and cell damage, thereby aggravating the disease. After treatment with Baicalin, blood viscosity was obviously improved, suggesting that Baicalin is able to improve microcirculation. At present, no reports on the effect of Baicalin in improving blood viscosity are seen in domestic or foreign literature.

To sum up, the release of a large number of inflammatory mediators and various active substances in SAP could induce the morphological alterations and functional disturbance of blood vessels and blood flow. In this study, our results shows that Baicalin can exert therapeutic effects on SAP through reducing the mortality rates of SAP rats, lowering the contents of inflammatory mediators and active substances in blood, improving microcirculation and mitigating pancreatic injury. Therefore, Baicalin, as a new drug, has good prospects in the treatment of SAP.

5 References

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Table 1 Comparision of pathological scores, PAF, amylase, TXB2, PGE2 IL-1β and ascites/body weight ratio ($M(Q_R)$)

Indexes	Sham-operated group			Model control group			Baicalin treated group		
	3h	6h	12h	3h	6h	12h	3h	6h	12h
pathological scores	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	7.00*** (3.00)	7.00*** (3.00)	10.00 *** (2.00)	6.00*** (2.00)	6.00 *** (2.00)	8.50***++ (2.00)
ascites/body weight ratio	0.35 (0.14)	0.40 (0.23)	0.39 (0.20)	2.17 *** (1.04)	3.20 *** (1.03)	3.26 *** (1.12)	1.72***+ (0.71)	1.83***+++ (0.96)	2.60***+ (1.33)
amylase	1460 (579)	1648 (493)	1714 (286)	5675*** (1243)	6632*** (3121)	10164*** (3752)	4878***+ (1723)	4973*** (3380)	7899***++ (1002)
IL-1β	5.32 (1.51)	4.51 (1.88)	5.31 (1.53)	28.42*** (13.26)	29.15*** (9.63)	19.33*** (16.86)	20.39***++ (11.28)	18.26***+++ (6.95)	16.15*** (7.70)
PAF	2.01 (0.89)	2.39 (0.68)	2.86 (0.50)	8.53*** (5.29)	9.94*** (8.53)	10.06*** (7.47)	8.29*** (3.62)	7.39*** (3.24)	9.30*** (3.19)
TXB_2	74367 (28685)	81460 (70585)	75891 (64452)	444864 *** (459803)	387872*** (336106)	330079 *** (365180)	411537*** (255318)	233710 ***+ (237615)	334108*** (202589)
PGE_2	7349 (4221)	6994 (5715)	7485 (4555)	27628*** (5435)	27125 *** (6257)	28125*** (16993)	29017*** (7143)	29910*** (15511)	29451*** (8929)

Note: compare to sham-operated group,* P<0.05,**P<0.01,***P<0.001; compare to model control group, +P<0.05,++P<0.01,+++P<0.001

Table 2 Comparision of PLA_2 among groups ($\overline{X} \pm S$)

Groups	3h	6h	12h
Sham-operated group	14.62±3.02	17.49±3.82	19.02±5.07
Model control group	76.09±16.70	101.46±14.67*	105.33±18.10*
Baicalin treated group	56.25±22.43	67.91±20.61*+	66.86±22.10*+

Note: compare to sham-operated group, *P<0.001; compare to model control group,+ P<0.001

Table 3 Comparison of blood viscosity parameters ($\overline{X} \pm S$)

Indexes	Sham-operated group		Model control group		Baicalin treated group	
	3h	12h	3h	12h	3h	12h
high-shear whole blood viscosity (200/s) (mPa.s)	2.88±0.53	2.98±0.55	4.12±0.73**	4.69±0.72***	3.60±1.32	3.80±0.77**++
whole blood viscosity (30/s)(mPa.s)	4.21±0.88	4.84±0.91	6.18±1.10***	7.03±1.53***	5.42±1.75*	5.51±1.44
low-shear whole blood viscosity (5/s)(mPa.s)	7.48±1.27	8.12±1.69	11.54±3.34***	11.57±2.42	9.54±2.17	10.65±3.40*
low-shear whole blood viscosity (1/s)(mPa.s)	13.28±2.83	16.14±3.16	32.07±12.23***	30±8.65***	20.83±5.68*++	21.67±6.31++

Note: compare to sham-operated group, * P<0.05, **P<0.01, ***P<0.001; compare to model control group, +P<0.05,++P<0.01,+++P<0.001